

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization  
International Bureau



(43) International Publication Date  
28 March 2002 (28.03.2002)

PCT

(10) International Publication Number  
**WO 02/24912 A2**

- (51) International Patent Classification<sup>7</sup>: **C12N 15/12**, 5/10, 5/20, C12Q 1/68, C07K 14/715, 16/18, G01N 33/68, 33/53, A61K 38/17
- (21) International Application Number: PCT/US01/29576
- (22) International Filing Date:  
21 September 2001 (21.09.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:  
60/234,583 22 September 2000 (22.09.2000) US  
60/245,495 3 November 2000 (03.11.2000) US  
09/919,162 31 July 2001 (31.07.2001) US
- (71) Applicant (for all designated States except US): **LUDWIG INSTITUTE FOR CANCER RESEARCH [CH/US]**; 605 Third Avenue, New York, NY 10158 (US).
- (72) Inventors; and
- (75) Inventors/Applicants (for US only): **RENAULD, Jean-Christophe [BE/BE]**; Avenue Hippocrate 74, UCL 7459, B- Brussels (BE). **DUMOUTIER, Laure [BE/BE]**; Avenue Hippocrate 74, UCL 74.59, B- Brussels (BE).
- (81) Designated States (national): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).
- Published:**  
— without international search report and to be republished upon receipt of that report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.



WO 02/24912 A2

A4 - 09/728,911

(54) Title: ISOLATED NUCLEIC ACID MOLECULES WHICH ENCODE A SOLUBLE IL-TIF/IL-22 RECEPTOR OR BINDING PROTEIN WHICH BINDS TO IL-TIF/IL-22, AND USES THEREOF

(57) Abstract: The invention relates to soluble proteins which bind to the molecule known as IL-TIF/IL-22. The proteins can antagonize the effect of IL-TIF/IL-22 on target cells. The nucleic acid molecules encoding the proteins, and uses of the protein, are also described.

**ISOLATED NUCLEIC ACID MOLECULES WHICH  
ENCODE A SOLUBLE IL-TIF/IL-22 RECEPTOR OR BINDING  
PROTEIN WHICH BINDS TO IL-TIF/IL-22, AND USES THEREOF**

**RELATED APPLICATIONS**

This application claims priority of Serial No. 09/919,162 filed July 30, 2001, and of provisional applications 60/245,495 filed November 3, 2000, and 60/234,583 filed September 22, 2000, all of which are incorporated by reference in their entirety.

**FIELD OF THE INVENTION**

This invention relates to newly isolated nucleic acid molecules, proteins and their uses. More specifically, it relates to a soluble protein which binds to the molecule referred to as IL-TIF/IL-22 or as it will be referred to hereafter "IL-22BP" or "IL-22 binding protein." The proteins of the invention inhibit TIF/IL-22 by binding thereto, and inhibiting IL-TIF/IL-22's effect on cells.

**BACKGROUND AND PRIOR ART**

The last decade has seen knowledge of the immune system and its regulation expand tremendously. One area of particular interest has been that of research on the proteins and glycoproteins which regulate the immune system. One of the best known families of these molecules are the cytokines. These are molecules which are involved in the "communication" of cells with each other. The individual members of the cytokine family have been found to be involved in a wide variety of pathological conditions, such as cancer and allergies. Whereas sometimes the cytokines are involved in the pathology of the condition, they are also known as being therapeutically useful.

Interleukins are one type of cytokine. The literature on interleukins is vast. An exemplary, but by no means exhaustive listing of the patents in this area includes U.S. Patent No. 4,778,879 to Mertelsmann et al.; U.S. Patent No. 4,490,289 to Stern; U.S. Patent No. 4,518,584 to Mark et al.; and U.S. Patent No. 4,851,512 to Miyaji et al., all of which involve interleukin-2 or "IL-2." Additional patents have issued which relate to

interleukin-1 ("IL-1"), such as U.S. Patent No. 4,808,611 to Cosman. The disclosure of all of these patents are incorporated by reference herein. More recent patents on different interleukins include U.S. Patent Nos. 5,694,234 (IL-13); 5,650,492 (IL-12); 5,700,664, 5,371,193 and 5,215,895 (IL-11); 5,728,377, 5,710,251, 5,328,989 (IL-10); 5,580,753, 5,587,302, 5,157,112, 5,208,218 (IL-9); 5,194,375, 4,965,195 (IL-7); 5,723,120, 5,178,856 (IL-6), and 5,017,691 (IL-4). Even a cursory review of this patent literature shows the diversity of the properties of the members of the interleukin family. One can assume that the larger cytokine family shows even more diversity. See, e.g., Aggarwal et al., ed., *Human Cytokines: Handbook For Basic And Clinical Research* (Blackwell Scientific Publications, 1992), Paul, ed., *Fundamental Immunology* (Raven Press, 1993), pg 763-836, "*T-Cell Derived Cytokines And Their Receptors*", and "*Proinflammatory Cytokines and Immunity*," and Thomson, ed. "*The Cytokine Handbook*" (1998, Academic Press). All cited references are incorporated by reference.

The relationships between various cytokines are complex. As will be seen from the references cited herein, as the level of a particular cytokine increases or decreases, this can affect the levels of other molecules produced by a subject, either directly or indirectly. Among the affected molecules are other cytokines.

The lymphokine IL-9, previously referred to as "P40," is a T-cell derived molecule which was originally identified as a factor which sustained permanent antigen independent growth of T4 cell lines. See, e.g., Uyttenhove et al., *Proc. Natl. Acad. Sci.* 85: 6934 (1988), and Van Snick et al., *J. Exp. Med.* 169: 363 (1989), the disclosures of which are incorporated by reference, as is that of Simpson et al., *Eur. J. Biochem.* 183: 715 (1989).

The activity of IL-9 was at first observed on restricted T4 cell lines, failing to show activity on CTLs or freshly isolated T cells. See, e.g., Uyttenhove et al., *supra*, and Schmitt et al., *Eur. J. Immunol.* 19: 2167 (1989). This range of activity was expanded when experiments showed that IL-9 and the molecule referred to as T cell growth Factor III ("TCGF III") are identical to MEA (Mast Cell Growth Enhancing Activity), a factor which potentiates the proliferative response of bone marrow derived mast cells to IL-3, as

is described by Hültner et al., *Eur. J. Immunol.* 19: 2167 (1989) and in U.S. Patent No. 5,164,317, the disclosures of both being incorporated by reference herein. It was also found that the human form of IL-9 stimulates proliferation of megakaryoblastic leukemia. See Yang et al., *Blood* 74: 1880 (1989). Recent work on IL-9 has shown that it also supports erythroid colony formation (Donahue et al., *Blood* 75(12): 2271-2275 (6-1990)); promotes the proliferation of myeloid erythroid burst formation (Williams et al., *Blood* 76: 306-311 (1990)); and supports clonal maturation of BFU-E's of adult and fetal origin (Holbrook et al., *Blood* 77(10): 2129-2134 (1991)). Expression of IL-9 has also been implicated in Hodgkins's disease and large cell anaplastic lymphoma (Merz et al., *Blood* 78(8): 1311-1317 (1990)). Genetic analyses of mice that were susceptible or resistant to the development of bronchial hyperresponsiveness have unraveled a linkage with the IL-9 gene as well as a correlation between IL-9 production and susceptibility in this model (Nicolaides et al., *Proc. Natl. Acad. Sci. USA*, 94, 13175-13180, (1997)). Human genetic studies also point to the IL-9 and IL-9R or "IL-9 receptor" genes as candidates for asthma therapy (Doull et al., *Am. J. Respir. Crit. Care Med.*, 153, 1280-1284, (1996); Holroyd et al., *Genomics* 52, 233-235, (1998)). IL-9 transgenic mice allowed for the demonstration that increased IL-9 expression results in lung mastocytosis, hypereosinophilia, bronchial hyperresponsiveness and high levels of IgE (Temann et al., *J. Exp. Med.* 188, 1307-1320 (1998); Godfraind et al., *J. Immunol.* 160, 3989-3996 (1998); McLane et al., *Am. J. Resp. Cell. Mol.* 19:713-720 (1999)). Taken together, these observations strongly suggest that IL-9 plays a major role in this disease. Additional work has implicated IL-9 and muteins of this cytokine in asthma and allergies. See, e.g. PCT Application US96/12757 (Levitt, et al.), and PCT US97/21992 (Levitt, et al.), both of which are incorporated by reference..

IL-9 is known to affect the levels of other molecules in subjects. See Louahed et al., *J. Immunol.* 154: 5061-5070 (1995), Demoulin et al., *Mol. Cell. Biol.* 16: 4710-4716 (1996), both incorporated by reference. It will be recognized that the molecules affected have their own functions in biological systems. For example, Demoulin et al. show that many of the known activities of IL-9 are mediated by activation of STAT transcription

factors. As such, there is continued interest in trying to identify molecules whose presence and/or level is affected by other molecules, such as signal transduction molecules and cytokines.

A new member of the interleukin family is described in, e.g., U.S. Patent Application Serial No. 09/419,568, filed October 18, 1999, and incorporated by reference in its entirety. Also see Dumoutier, et al., "Human interleukin-10 related T cell derived inducible factor molecular cloning and function characterization as a hepatocyte stimulating factor," *Proc. Natl. Acad. Sci. USA* 97(18): 10144-10149 (2000), also incorporated by reference. Also see Dumoutier, et al., *J. Immunol* 164:1814 (2000), and Dumoutier, et al., *Genes Immunol* 1:488 (2000), both of which are incorporated by reference. Also see Serial No. 09/626, 627 filed July 27, 2000, incorporated by reference. Dumoutier, et al., *Proc. Natl. Acad. Sci.* 97(18): 10144-10149 (2000) also suggest that this new molecule, IL-TIF/IL-22, induces acute phase reactant production by liver cells, *in vitro*, and *in vivo*. Xie, et al., *J. Biol. Chem* 27:31335-31339 (2000), have suggested that this molecule be renamed as IL-22. Xie et al also teach that the receptor for this molecule consists of two chains, each of which bind to the molecule. These chains are referred to as "CRF 2-4" and "CRF 2-9." The former is also referred to as "IL-10RB" because it is required for IL-10 signalling. See, e.g., Kotenko, et al., *EMBO J* 16:5894 (1997).

The second chain, CRF 2-9, was originally considered to be an orphan receptor. This chain is also known as "ZCYTOR 11," but Xie, et al., *supra*, have proposed it be renamed "IL-22R". Due to their structure, both chains are considered to belong to the class II cytokine receptor family (Kotenko, et al., *Oncogene* 19:2557 (2000)), which consists of 8 members of known function (i.e., two pairs of two receptor subunits for type I interferons (IFN- $\alpha$ , IFN- $\beta$ , IFN- $\omega$ , IFN- $\tau$ ) and type II (IFN- $\gamma$ ) interferon, IL-10R, tissue factor, and the two chains referred to *supra*. At least one orphan receptor, referred to as "CRF 2-8," is also a member of the family. These receptors are related by their extracellular domains, which have tandem fibronectin type III (FNIII) domains. Four of the genes encoding these proteins, i.e., "IFNAR1," "IFNAR2," "IL10R2" and "IFNGR2,"

are located on human chromosome 21. The IFNGR1 and CRF2-8 genes map to chromosome 6, IL22R is located on chromosome 1, and IL10R1 is on chromosome 11.

Additional work on these molecules can be found in, e.g., International Patent Application Number PCT/US00/11479 (Publication Number WO 00/65027) and International Parent Application Number PCT/US99/11644 (Publication Number WO 99/61667). Also see International Patent Application Number PCT/US00/32703, publication number WO/01/40467, describing "ZCYTOR16."

A nucleic acid molecule has now been identified, which encodes another molecule which binds IL-TIF/IL-22 and is referred to as IL-22 binding protein (IL-22BP). The protein which the nucleic acid molecule encodes serves to inhibit the effect that IL-TIF/IL-22 has on target cells. Further, a second form of the nucleic acid molecule has been identified as a splice variant of the first. This second molecule contains an additional 96 nucleotides, and encodes an additional 32 amino acids.

These, as well as other features of the invention, will be seen in the disclosure which follows.

#### **DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS**

##### **EXAMPLE 1**

This example describes experiments which were carried out to identify potential new members of the class II cytokine receptor family. Receptors for the interferons, and for IL-10, are members of this family.

The amino acid sequence of the extracellular domain of human IL-10R was used to screen the database of the Sanger Center ([http://www.sanger.ac.uk/c/cgi-bin/nph-Blast\\_server;html](http://www.sanger.ac.uk/c/cgi-bin/nph-Blast_server;html)), using TBLASTN software.

Two short regions of homology were identified in a BAC clone from chromosome 6q24 (Genbank Accession Number AL 05337), about 40 kilobases from the known IFNGR-1 gene.

The first fragment showed 40% amino acid identity with residues 63-119 of IL-10R, while the other, located 3kb upstream, showed 47% identity with residues 29-47.

Once the BAC sequence was identified, it was analyzed further, using the NIX analysis program found at <http://www.hgmp.mrc.ac.uk/Registered/Webapp/nix/>, incorporated by reference. The software predicted a gene comprising 5 exons, stretching over about 16 kilobases, with the last exon corresponding to several EST sequences.

## EXAMPLE 2

These experiments were designed to determine the pattern of tissue distribution of the molecule identified in example 1, supra.

Total RNA was isolated from samples taken from various organs using guanidium isothiocyanate lysis and CsCl gradient centrifugation, following Ausubel, et al., Current Protocols In Molecular Biology (1993), incorporated by reference. Samples of RNA (5ug), were reverse transcribed with an oligo(dT) primer, and the resulting cDNA was amplified via PCR. Specifically, samples corresponding to 5ng of total RNA were amplified using

agggtacaat ttcagtcccg a

(sense, SEQ ID NO: 1)

and

cggcgtcatg ctccattctg a

(antisense, SEQ ID NO: 2). The annealing temperature was 55°C. Resulting PCR products were analyzed in ethidium bromide stained, 1% agarose gels.

Strongest expression was found in breast tissue, and a clear signal was also detected in lungs and the intestinal tract (i.e., stomach and colon). Skin, testis, brain, heart, and thymus tissue were also positive, at lower levels, and not in all samples tested. There was no detectable expression in prostate, bladder, kidney, ovary, muscle, bone marrow, liver or uterine tissue.

One noteworthy feature of these results was the identification of a second band in some tissue samples, such as skin and lungs. The significance of this second band is discussed infra.

**EXAMPLE 3**

These experiments describe work in amplifying full length cDNA for the materials described supra.

Breast tissue RNA was prepared, as described supra, and was amplified via RT-PCR, as described supra, using:

tgaacagtca cactcgagac catgatgc

(sense; SEQ ID NO: 3),

and

catcctgttc tcgaggagct ttaga

(antisense; SEQ ID NO: 4).

These primers contain mutations which introduce an XhoI site to permit direct cloning into pCEP4 plasmid described infra. A cDNA molecule was identified which consisted of a 696 nucleotide open reading frame that encoded a protein of 230 or 231 amino acids, with a calculated molecular weight of about 27kd. The complete nucleotide sequence included 2271 nucleotides, with the "ORF" at nucleotides 113-805, or 110-805. The nucleotide sequence, and the predicted amino acid sequence, are presented as SEQ ID NOS: 5 and 6. Note that there are two potential start codons adjacent to each other, so that the protein may begin with Met or Met Met.

Analysis of the predicted protein reveals a stretch of hydrophobic amino acids at the N-terminus, compatible with a signal peptide. There was significant homology to the extracellular domains of members of the IL-10 receptor family; however, the molecule under consideration lacked a hydrophobic transmembrane domain, suggesting it is a secreted protein.

When the deduced amino acid sequence was aligned with other proteins, 33% amino acid identity with the extracellular domain of IL-22R was found, as was 34% with orphan receptor CRF 2-8. Lower sequence identity was found with IL-10R (29%), CRF2-4/IL-10R $\beta$  (30%), tissue factor (26%) and the four interferon receptor chains (23-25%). The predicted mature form of the protein contains 4 cysteine residues. These are



conserved in most members of the class II cytokine receptors. Additionally, the structure of the gene, as deduced from the information presented herein, is of one that contains 5 exons, the first of which encodes the signal peptide, and the following four of which encode the mature protein.

#### EXAMPLE 4

The data developed supra showed that the molecule of interest had highest homology to the extracellular domain of IL-22R. Experiments were therefore designed to determine if the molecule bound to IL-TIF/IL-22.

A series of IL-22BP fusion proteins were made. The first, referred to as "IL-TIFR-Ig," was produced by first amplifying the full length open reading frame of the IL-22BP molecule referred to supra, using the following, mutated antisense primer:

ccaactcca    tgatcaatgg    aattccaca    catctct

(SEQ ID NO. 7)

This primer serves to introduce a BclI site into the stop codon of the ORF. In addition, a region comprising the hinge, CH2 and CH3 domains of murine IgG3 isotype heavy chain was amplified, using the known IgG3 anti-TNP hybridoma C3110. The following primers were used:

aagactgagt    tgatcaagag    aatcgagcct    aga

(sense, SEQ ID NO. 8)

and

aatgtctaga    tgctgttctc    atttacc

(antisense, SEQ ID NO. 9)

These primers also contain BclI and XbaI sites for cloning.

Following amplification, both PCR products were digested, and cloned into pCEP4 plasmid, under control of CMV promoter, as described supra.

Clones were sequenced, using standard methodologies. These were then used to transfect HEK293 cells transiently, also as described supra. In brief, cells were seeded in 6 well plates, at  $3 \times 10^5$  cells/well, one day prior to transfection. Standard, lipofectamine

methodologies were used, using 2 ug of plasmid DNA. After transfection, cells were incubated in 1.5 ml of normal medium for 3 days.

In similar fashion, a fusion protein of IL-22R and the IgG3 Fc fragment was generated, known as IL-22R-Ig. These two fusion proteins were used together with a control fusion protein, i.e. IL-9-Ig, which had been made previously.

Assays were then carried out by coating polystyrene plates with either 0.083 mg/ml of recombinant human IL-TIF/IL-22, or 0.2 mg/ul bovine serum albumin, in 20mm Tris. Glycine buffer containing 30 mm NaCl, pH 9.2, overnight, at 4°C. Following washing in PBS buffer plus Tween 20 ( $10^{-4}$ ) plates were blocked with PBS plus 1% BSA for two hours, and then 50  $\mu$ l of supernatant from transiently transfected HEK293 cells was added. Plates were then incubated for 2 hours, at 37°C. Any bound fusion protein was detected, using murine anti-Ig polyclonal antibodies coupled to peroxidase. Detection was carried out using the peroxidase substrate "TMB", or (3, 3', 5, 5' - tetramethylbenzidine), and stopped by 20 $\mu$ l H<sub>2</sub>SO<sub>4</sub>.

The results indicated that IL-22BP-Ig and IL-22R-Ig both bind to IL-TIF/IL-22, but not bovine serum albumin. Supernatants of mock transfected cells, or IL-9-Ig did not detectably bind IL-TIF/IL-22.

#### **EXAMPLE 5**

These experiments describe studies designed to assess whether the protein of the invention was able to block IL-TIF/IL-22 activity.

To test this, the cell lines H4IIE and HT-29, referred to supra, were used. It is known that H4IIE responds to IL-TIF/IL-22 by activation of STAT transcription factors, and acute phase reactant production. The HT-29 cell line shows STAT-3 activation. STAT activation by IL-TIF/IL-22 can be measured, in both cell lines, via the use of a luciferase reporter construct which includes 5 STAT binding sites, plus a minimal TK promoter. See Dumoutier, et al.; Proc. Natl. Acad. Sci. USA, 97:10144 (2000), incorporated by reference.

The construct "pGRR5" was used. This construct contains 5 copies of the STAT binding site of the FcγR1 gene, upstream of a luciferase gene under control of a tk promoter. As an internal control, the vector pRL-TK was used. This construct contains the renilla luciferase gene under the control of the tk promoter.

The H4IIE and HT-29 cells were electroporated with 15 μg of pGRR5 and 1 μg pRL-TK (250V, 192 Ω, 1,200 μF), and were then seeded at 4x10<sup>5</sup> cells/well. RAW 264.7 cells were transfected in the same way, the only difference being the resistance used (74Ω). This cell line was used to determine the effect of the IL-22BP material on IL-10.

The transfected H4IIE or HT-29 cells were then stimulated with a preincubated (1 hour) mixture of recombinantly produced IL-TIF/IL-22, at varying concentrations and 5% supernatant (from HEK293 cells that had been transfected with the cDNA described herein), or a preincubated mixture of the IL-TIF and 5% supernatant from mock transfected cells. After two hours, luciferase activity was measured either in pelleted or lysed cells, or directly in plated cells, using a commercially available assay.

The results indicated that the STAT-activating activity of IL-TIF/IL-22 (at 4ng/ml), was blocked completely when combined with supernatants from cells transfected by constructs encoding the IL-22BP protein or fusion proteins, described supra. This was the case for both H4IIE and HT-29 cells. In contrast, when IL-6 was used in place of IL-TIF/IL-22, there was no effect. Nor was IL-10 activity affected by pre-incubation with IL-IL-22BP protein or fusion protein.

#### **EXAMPLE 6**

Novick, et al, Cytokine 4:6 (1992), have shown that soluble IL-6 receptor can increase the sensitivity of cells to subliminal concentrations of its ligand. Studies were therefore carried out, in parallel to those presented supra, testing low (<25 ng/ml), and high (50-200 ng/ml) concentrations of IL-TIF/IL-22 in H4IIE cells. It was found that, at the low concentrations, STAT activation was blocked completely by IL-22BP but IL-22BP failed to block STAT activation in H4IIE cells, when high concentrations of IL-TIF

were used. Decreasing the concentration of IL-22BP led to a loss of inhibitory effect, but did not reveal any potentiating activity for IL-TIF/IL-22.

#### EXAMPLE 7

Fernandez-Botran, et al., J. Exp. Med 174:673 (1991) have shown that the soluble and transmembrane forms of the IL-4 receptor have similar association rates, but the soluble form has a higher dissociation rate. This indicates that the complexes formed by IL-4 and the IL-4 binding protein ("IL-4BP") must be transient and reversible allowing the ligand to dissociate from one soluble receptor and become available for binding to another soluble receptor or to a membrane receptor from which it would dissociate more slowly. Experiments were carried out to determine if the protein of the invention exhibited the same property and thus delay rather than inhibit IL-TIF/IL-22 action.

It was found that the effect of IL-TIF/IL-22 on STAT-activation in HT-29 cells reached its peak after 4-6 hours, and decreased dramatically at 24 hours but the receptor of the invention had the same inhibitory effect throughout the assay, indicating that it could not delay IL-TIF/IL-22 activity in vitro.

#### EXAMPLE 8

Example 2, supra, referred to the identification of a second band in some tissue samples. The band was excised, and sequenced using an automated, fluorescence based system, and art recognized methods. The sequence, set forth at SEQ ID NO. 10, includes an additional 96 nucleotides, resulting in 32 additional amino acids in the predicted protein (SEQ ID NO. 11). The ORF for SEQ ID NO: 10 extends from nucleotide 109 or 112 to nucleotide 900. As with SEQ ID NOS: 10 & 11, there are two possible start codons.

The preceding examples disclose the aspects of this invention, including isolated nucleic acid molecules which encode a soluble, receptor-like antagonist of IL-TIF/IL-22 such as those with the amino acid sequence of the protein encoded by the nucleotide sequences set forth in SEQ ID NO: 5 or 10. It will be appreciated by one of ordinary skill that the degeneracy of the genetic code facilitates the preparation of nucleic acid

molecules which may not be identical to the nucleotide sequence of SEQ ID NO: 5 or 10, but which encode the same protein. Of course, SEQ ID NO: 5 or 10 are preferred embodiments of this invention, but other embodiments are also a part of the invention. Genomic DNA, complementary DNA, and RNA, such as messenger RNA, are all to be included therein. Isolated nucleic acid molecules from other animal species, including other mammals, are also a part of the invention. A preferred aspect of the invention are isolated nucleic acid molecules whose complements hybridize to SEQ ID NO: 5 or 10 under stringent conditions. "Stringent conditions," as used herein, refer, for example, to hybridization at 65°C in buffer (3.5xSSC), 0.02% Ficoll, 0.02% polyvinylpyrrolidone, 0.02% bovine serum albumin, 25mM NaH<sub>2</sub>PO<sub>4</sub> (pH 7), 0.1% SDS, 2mM EDTA, followed by a final wash at 2xSSC, room temperature and then 0.1xSSC/0.2xSDS at temperatures as high as, e.g., about 65°C. More stringent conditions, such as 0.1xSSC, can also be used. These nucleic acid molecules encode proteins, such as those with amino acid sequences set forth at SEQ ID NO: 6 or 11. The soluble, receptor-like antagonist of this invention may be found in glycosylated or non-glycosylated, sulfated and non-sulfated forms and so forth. Also a part of the invention are isolated nucleic acid molecules which encode proteins having 30% or more, preferably 45% or more, more preferably 50% or more, 60% or more, 70% or more, 80% or more, or 90% or more, and most preferably 95% or more amino acid identity with an amino acid sequence of a protein encoded by SEQ ID NO: 5 or 10.

Amino acid sequence identity may be determined using the algorithm GAP (Genetics Computer Group, Madison, WI) or identity. GAP uses the Needleman and Wunsch algorithm to align two complete sequences that maximizes the number of matches and minimizes the number of gaps. Generally, the default parameters are used, with a gap creation penalty = 12 and gap extension penalty = 4. Use of GAP may be preferred but other algorithms may be used, e.g. BLAST (which uses the method of Altschul et al. (1990) *J. Mol. Biol.* 215: 405-410), FASTA (which uses the method of Pearson and Lipman (1988) *PNAS USA* 85: 2444-2448), or the Smith-Waterman

algorithm (Smith and Waterman (1981) *J. Mol Biol.* 147: 195-197), generally employing default parameters.

Also a part of the invention are expression vectors which include the nucleic acid molecules of the invention, operably linked to a promoter, so as to facilitate expression of the DNA. It is well within the skill of the artisan to prepare such vectors.

The vectors, as well as the nucleic acid molecules *per se*, can be used to prepare recombinant cells, such as isolated recombinant cells, be these eukaryotic or prokaryotic, wherein either an expression vector or the nucleic acid molecule itself is incorporated therein. *E. coli* cells, COS cells, CHO cells, Sf9 cells etc., are all examples of types of cells which may be used in accordance with this aspect of the invention.

Generally, nucleic acid molecules employed to produce a polypeptide or fragment thereof according to the present invention are provided as isolates, in isolated and/or purified form, or free or substantially free of material with which they are naturally associated, such as free or substantially free of nucleic acid molecules flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid molecules may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA.

Nucleic acid molecules encoding the peptides or polypeptides of the present invention may be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook and Russell "Molecular Cloning, A Laboratory Manual", Third Edition, Cold Spring Harbor Laboratory Press, 2001, and Ausubel et al, Current Protocols in Molecular Biology, John Wiley and Sons, 1992, or later edition thereof).

In order to obtain expression of a nucleic acid molecule of the invention, this may be incorporated in a vector having one or more control sequences operably linked to the nucleic acid molecule to control its expression. Vectors may be chosen or constructed. They may contain appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate, e.g. nucleotide sequences so that the polypeptide or

peptide is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide or peptide produced in the host cell is secreted from the cell. Vectors may be plasmids, viral e.g. phage, or phagemid, as appropriate. Encoded product may then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the product is produced and recovering the product from the host cells or the surrounding medium.

A further aspect provides a method which includes introducing a nucleic acid molecule of the invention into a host cell. The introduction, which may (particularly for *in vitro* introduction) be generally referred to without limitation as "transformation" or "transfection", may employ any available technique. For eukaryotic cells, suitable techniques may include calcium phosphate transfection, DEAE-Dextran, electroporation, liposome-mediated transfection and transduction using retrovirus or other virus, e.g. vaccinia or, for insect cells, baculovirus. For bacterial cells, suitable techniques may include calcium chloride transformation, electroporation and transfection using bacteriophage. As an alternative, direct injection of the nucleic acid could be employed. Marker genes such as antibiotic resistance or sensitivity genes may be used in identifying clones containing nucleic acid of interest, as is well known in the art.

The introduction may be followed by causing or allowing expression from the nucleic acid molecule, e.g. by culturing host cells (which may include cells actually transformed although more likely the cells will be descendants of the transformed cells) under conditions for expression of the gene, so that the encoded product is produced. If the product as expressed is coupled to an appropriate signal leader peptide, it may be secreted from the cell into the culture medium. Following production by expression, a product may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in an assay or test as disclosed herein.

Note also that expression may also be carried out in *in vitro* systems, e.g. reticulocyte lysate.

Following production of a polypeptide or peptide as identified herein, it may be tested for ability to bind IL-TIF/IL-22 and/or for ability to modulate binding of IL-22BP to IL-TIF/IL-22.

A further aspect of the present invention provides a host cell containing a heterologous nucleic acid molecule encoding a polypeptide or peptide as disclosed herein. The nucleic acid of the invention may be integrated into the genome (e.g. chromosome) of the host cell. Integration may be promoted by inclusion of sequences which promote recombination with the genome, in accordance with standard techniques. The nucleic acid may be on an extra-chromosomal vector within the cell, or otherwise identifiably heterologous or foreign to the cell.

Proteins encoded by the above referenced nucleic acid molecules, preferably in isolated form, are another feature of this invention. By "protein" is meant both the immediate product of expression of the nucleic acid molecules, glycosylated forms of it, forms of the molecule following peptide signal cleavage, such as mature and/or processed forms of the protein, as well as multimeric forms, such as dimers, trimers, and so forth. Also a part of the invention are multimers, such as dimers, which contain at least one protein molecule of the invention, and at least one, different protein molecule. These multimers may be homomeric or heteromeric, such as heteromeric forms that include at least one molecule of a different soluble receptor, a transmembrane receptor, and so forth. Such multimers may bind only a single specific ligand. Also a part of the invention are complexes of the IL-22BP and a ligand, which then act as heteromeric cytokines in transmembrane receptors. Such structures parallel, e.g., the structure of IL-12. Also a feature of this invention is a protein consisting of the sequence set forth in SEQ ID NO: 6 or 11. Also included as a feature of this invention are proteins that are essentially identical to the sequence in SEQ ID NO: 6 or 11 having only conservative amino acid substitutions. Also included as a feature of the inventions are constructs, such as fusion proteins, where all or a part of the proteins described supra are linked in some fashion, e.g., to a "fusion partner" at least one additional protein or peptide, or amino acid sequence. The "fusion partner" may be, for example, a molecule which provides a



recognizable signal, either directly or indirectly, such as a FLAG peptide,  $\beta$ -galactosidase, luciferase, an Fc immunoglobulin, a fluorescent protein, such as "GFP" (green fluorescent protein), and so forth. Other labels, such as radiolabels, particles, other enzymes, metals such as gold sols, may also be used. These fusion partners are preferably joined to the molecule which is described supra at the N - and/or C- terminus of the protein; however, it is to be understood that there are many techniques known for joining molecules to amino acids, and any and all of these methodologies can produce constructs which are a part of the invention.

The individual protein molecules of the invention will preferably have a molecular weight of from about 23 to about 40 kilodaltons as determined by SDS-PAGE. In multimeric forms, the molecular weight of the complex will, of course, vary, but the individual molecules contained therein will each have a molecular weight of about 23-40 kilodaltons, as determined by SDS-PAGE. These molecular weights, it is to be understood, refer to monomeric proteins. Glycosylated monomers will have higher molecular weights, e.g., up to at least about 40-50 kilodaltons.

The proteins preferably consist of at least about 180 and no more than about 300 amino acids. More preferably, the protein consists of about 230-275, more preferably 230-268, most preferably 231-263 amino acids. Preferably, the amino acids sequences consists of or comprises all or part of the amino acid sequences encoded by SEQ ID NO: 6 or 11. Such binding proteins can be produced via, e.g., transforming host cells with one or more nucleic acid molecules or expression vectors in accordance with the invention, culturing the transformant, and then isolating the resulting, recombinant binding protein.

It will be appreciated by the skilled artisan that the proteins and peptides encoded by the above recited nucleic acid molecules are a feature of the invention, and may be used to produce antibodies, in accordance with standard protocols. Such antibodies, in monoclonal and polyclonal form, constitute a further feature of the invention as do fragments of said antibodies, chimeric forms, humanized forms, recombinant forms, hybridoma cell lines which produce the antibodies and so forth.

Antibody molecules directed to IL-22BP, especially a region involved in binding to IL-TIF/IL-22, are also provided by a further aspect of the present invention. Such antibody molecules are useful for inhibiting IL-22BP binding to IL-TIF/IL-22, also for purifying IL-22BP.

Antibody molecules may be obtained using techniques which are standard in the art. Methods of producing antibodies include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep or monkey) with the relevant polypeptide or a peptide fragment thereof. Antibody molecules may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage *et al.*, 1992, *Nature* 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunizing a mammal with a peptide or polypeptide, an antibody molecule may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibody molecules useful in accordance with the present invention may be modified in a number of ways. Indeed the term "antibody molecule" should be construed as covering antibody fragments and derivatives able to bind antigen. Examples of antibody fragments, capable of binding an antigen or other binding partner are the Fab fragment consisting of the VL, VH, Cl and CH1 domains; the Fd fragment consisting of the VH and CH1 domains; the Fv fragment consisting of the VL and VH domains of a single arm of an antibody; the dAb fragment which consists of a VH domain; isolated CDR regions and F(ab')<sub>2</sub> fragments, a bivalent fragment including two Fab fragments linked by a disulphide bridge at the hinge region. Single chain Fv fragments are also included.

Hybridomas capable of producing antibodies with desired binding characteristics are within the scope of the present invention, as are host cells, eukaryotic or prokaryotic, containing nucleic acid molecules encoding antibodies (including antibody fragments) and capable of their expression. The invention also provides methods of production of the antibody molecules including growing a cell capable of producing the antibody under conditions in which the antibody is produced, and preferably secreted. Such methods generally comprise isolation or purification of antibody molecules from the cells or culture medium.

The reactivities of antibody molecules on a sample may be determined by any appropriate means. Tagging with individual reporter molecules is one possibility. The reporter molecules may directly or indirectly generate detectable, and preferably measurable, signals. The linkage of reporter molecules may be directly or indirectly, covalently, e.g. via a peptide bond or non-covalently. Linkage via a peptide bond may be as a result of recombinant expression of a gene fusion encoding antibody and reporter molecule. The mode of determining binding is not a feature of the present invention and those skilled in the art are able to choose a suitable mode according to their preference and general knowledge.

Antibody molecules may also be used in purifying and/or isolating a polypeptide chain of the invention, or a peptide fragment, for instance following production of a polypeptide by expression from encoding nucleic acid. Antibody molecules may be useful in a therapeutic context (which may include prophylaxis) to disrupt binding of polypeptides or other molecules with a view to inhibiting the relevant biological function or activity.

Also a feature of the invention are immunogens, comprising all or a part of the amino acid sequence of protein molecules of the invention, preferably combined with an adjuvant, such as Complete or Incomplete Freund's Adjuvant. An immunogenic or antigenic fragment of IL-22BP useful for obtaining antibody molecules may comprise or consist of one or more epitopes of IL-22BP. Linear epitopes are generally 5-8 amino acids in length, and peptides consisting of or comprising one or more epitopes or

antigenic determinants of IL-22BP are provided as a further aspect of the invention. Portions of the protein sequences may be linked to other molecules, such as keyhole limpet hemocyanin, to render them more immunogenic.

A "fragment" of a polypeptide generally means a stretch of amino acid residues of at least about five contiguous amino acids, often at least about seven contiguous amino acids, typically at least about nine contiguous amino acids, more preferably at least about 13 contiguous amino acids, and, more preferably, at least about 20 to 30 or more contiguous amino acids. A peptide fragment may be 5, 6, 7, 8, 9 or 10, 5 to 10, 5 to 20, 10 to 20, 10-30, 20-30, 20-40, 30-40 or less than 40 amino acids in length.

As noted, peptides may be made recombinantly by expression from encoding nucleic acid. Peptides can also be generated wholly or partly by chemical synthesis. They can be readily prepared according to well-established, standard liquid or, preferably, solid-phase peptide synthesis methods, general descriptions of which are broadly available (see, for example, in J.M. Stewart and J.D. Young, *Solid Phase Peptide Synthesis*, 2nd edition, Pierce Chemical Company, Rockford, Illinois (1984), in M. Bodanzsky and A. Bodanzsky, *The Practice of Peptide Synthesis*, Springer Verlag, New York (1984); and Applied Biosystems 430A Users Manual, ABI Inc., Foster City, California), or they may be prepared in solution, by the liquid phase method or by any combination of solid-phase, liquid phase and solution chemistry, e.g. by first completing the respective peptide portion and then, if desired and appropriate, after removal of any protecting groups being present, by introduction of the residue X by reaction of the respective carbonic or sulfonic acid or a reactive derivative thereof.

A further aspect of the present invention provides a method of obtaining an antibody directed against and preferably specific for IL-22BP, the method comprising bringing a population or panel of antibody molecules of diverse binding specificity into contact with an IL-22BP polypeptide or an antigenic or immunogenic fragment thereof, and selecting one or more antibody molecules that binds the polypeptide or fragment thereof. Preferably an antibody molecule that binds the polypeptide or fragment thereof is tested for specificity of its binding for the polypeptide or fragment thereof, e.g. by

testing binding on a panel of unrelated antigens, for example by ELISA as is standard in the art. Preferably an antibody molecule specific for IL-22BP is identified.

A population of antibody molecules may for example be provided as a phage display library and brought into contact with the polypeptide or fragment thereof *in vitro*. Another of the various options available to the skilled person is to administer a peptide or polypeptide to a mammal in order to raise an immune response. Antibody molecules and/or cells producing antibody molecules can be taken or harvested from the animal or its serum, and tested for the desired property or properties.

Once obtained, an antibody molecule can be formulated into a composition comprising at least one additional component, such as a pharmaceutically acceptable excipient or carrier, and may be used as desired.

As noted, antibody molecules can be used, e.g., to determine if the proteins of the invention are present. This is a further feature of the invention, as is explained elsewhere herein.

It has been shown, in the examples, that the nucleic acid molecules of the invention encode proteins that block IL-TIF/IL-22 activity. Hence, a further feature of the invention is a method inhibiting IL-TIF/IL-22 activity, such as the activation of STAT transcription factors and acute phase reactant production by contacting a sample with an amount of the protein of this invention sufficient to inhibit or block the activity of IL-TIF/IL-22.

One could also use these molecules to test the efficacy of IL-9 agonists or antagonists when administered to a subject, such as a subject suffering from lymphoma, an immune system disorder such as an allergy, acquired immune deficiency syndrome, autoimmune diabetes, thyroiditis, or any of the other conditions described in, e.g., U.S. Patent No. 5,830,454; 5,824,551, and pending application Serial No. 08/925,348, filed on September 8, 1997 now allowed, all of which are incorporated by reference. The molecules can also be used to modulate the role of IL-9 in these and other conditions. To elaborate, since IL-9 induces IL-TIF/IL-22 and the proteins of this invention block the activity of IL-TIF/IL-22, the proteins of this invention are useful as IL-9 activity

modulators. Thus, a further aspect of the invention is a method to determine activity of endogenous IL-9, such as in situations where excess IL-9 activity is implicated, including asthma, allergies, and lymphomas. One can also block or inhibit IL-9 activity by blocking or inhibiting IL-TIF/IL-22 or IL-TIF/IL-22 activity, using the receptor-like antagonist of this invention. Examples of conditions which can be treated by the use of the protein or peptide of this invention are allergies, asthma, lymphoma, and so forth. The ability to regulate IL-9 activity is important in conditions such as those listed supra, as well as conditions such as apoptosis, including cortisol induced apoptosis, conditions involving the nuclear expression of BCL-3, since IL-9 is known to induce such expression, and so forth.

IL-TIF/IL-22 type molecules may either promote regeneration or inhibit differentiation of tissue types in which these molecules are active. IL-TIF/IL-22 molecules target various cancer and normal cell lines (i.e., mesangial and neuronal cells, as well as melanoma and hepatoma cells. See, e.g., US Patent Application 09/626,617 filed July 27, 2000, incorporated by reference). Hence, another feature of the invention is a method of treatment of a patient in need thereof wherein the proteins of this invention are used to inhibit the activity of IL-TIF/IL-22, in, e.g., neoplastic tissue, such as melanoma or hepatoma.

It will be clear to the skilled artisan that IL-TIF/ IL-22 can regulate the inflammatory response. A preferred aspect of this regulation is the modulation of the acute phase response by organs, such as the liver, by administering the receptor-like antagonist of this invention. See, e.g., Janeway et al., Immunobiology, (4<sup>th</sup> edition), incorporated by reference. Janeway explains that various cytokines such as IL-1, IL-6 and TNF- $\alpha$  activate hepatocytes to synthesize acute phase proteins, such as c-reactive protein, and mannan binding lectin, as well as those described in the examples, supra.

IL-TIF/IL-22 has a role in activating acute phase proteins. Thus another aspect of this invention is a method for reducing the production of acute phase proteins, stimulated by IL-TIF/IL-22, by administering an amount of the receptor-like antagonist of this

invention to a tissue sample or to a patient in need thereof, wherein said amount is sufficient to reduce production or activity of acute phase proteins.

Also a part of the invention are methods for regulating activity of IL-TIF/IL-22 by administering the receptor-like antagonist to regulate IL-TIF/IL-22 activity.

As noted, IL-22BP fragments that inhibit binding of the ligand to the receptor may be used, as may antibody molecules, and small molecules or other agents identified using for example an assay of the invention as disclosed herein. Accordingly, disclosure of aspects of the invention making use of IL-22BP in therapeutic, prophylactic or diagnostic contexts should be taken as disclosure of analogous aspects of the invention making use of any one or more of an IL-22BP fragment or fragments, an antibody molecule that binds and preferably is specific for IL-22BP, especially an antibody molecule that affects binding of IL-22BP to IL-TIF/IL-22, and an agent identified using an assay of the invention, able to modulate IL-22BP binding to IL-TIF/IL-22.

Whether a protein, polypeptide, peptide, antibody molecule, small molecule or other substance is to be employed for a therapeutic purpose, e.g. in treatment of a condition identified herein, in various further aspects, the present invention further provides a pharmaceutical composition, medicament, drug or other composition for such a purpose, the composition comprising one or more such substances, the use of such a substance in a method of medical treatment, a method comprising administration of such a substance to a patient, e.g. for treatment (which may include preventative treatment) of a medical condition, use of such a substance in the manufacture of a composition, medicament or drug for administration for such a purpose, e.g. for treatment of a medical condition, and a method of making a pharmaceutical composition comprising admixing such a substance with a pharmaceutically acceptable excipient, vehicle or carrier, and optionally other ingredients.

Whatever the substance used in a method of medical treatment of the present invention, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual

amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors.

Pharmaceutical compositions according to the present invention, and for use in accordance with the present invention, may include, in addition to active ingredient, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material will depend on the route of administration, which may be oral, or by injection, e.g. cutaneous, subcutaneous or intravenous.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included, as required.

Examples of techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Osol, A. (ed), 1980.

Instead of administering such substances directly, they may be produced in target cells by expression from an encoding nucleic acid introduced into the cells, e.g. from a viral vector or as "naked" DNA administered to the body. Nucleic acid encoding the substance e.g. a peptide able to modulate, e.g. interfere with, the interaction of IL-22BP



and IL-TIF/IL-22, may thus be used in methods of gene therapy, for instance in treatment of individuals, e.g. with the aim of preventing or curing (wholly or partially) a disorder.

A polypeptide or peptide of the invention can be used in assaying for agents and substances that, by affecting the association or interaction between IL-TIF/IL-22 and IL-22BP, modulate IL-TIF/IL-22 function *in vivo*. Formats that may be used in such assays are described in detail below, and may comprise determining binding between components in the presence or absence of a test substance and/or determining ability of a test substance to modulate a biological or cellular function or activity in which binding of IL-22BP to IL-TIF/IL-22 plays a role. Assay methods that involve determination of binding between components and the effect of a test substance on such binding need not necessarily utilize full-length wild-type polypeptide chains. For instance, fragments of IL-22BP that retain ability to bind IL-22 may be employed, and *vice versa*. Indeed, as discussed further below, fragments of the polypeptides themselves represent a category of putative inhibitors, that may be used to interfere with binding between polypeptides.

Assays employing polypeptides and fragments thereof according to the invention may take any of a variety of formats.

One further aspect of the present invention provides an assay method for a substance able to interact with the relevant region of IL-22BP that binds to IL-TIF/IL-22, or the relevant region of IL-TIF/IL-22 that binds IL-22BP, as the case may be, the method comprising:

- (a) bringing into contact an IL-22BP polypeptide or fragment thereof that binds IL-TIF/IL-22; or an IL-TIF/IL-22 polypeptide or a fragment thereof that binds IL-22BP, and a test compound; and
- (b) determining interaction or binding between said polypeptide or fragment thereof and the test compound.

A test compound found to interact with or bind to the relevant portion of the polypeptide may be tested for ability to modulate, e.g. disrupt or interfere with, binding between IL-TIF/IL-22 and IL-22BP, or to modulate an activity mediated by IL-TIF/IL-22.

In a further aspect the present invention provides an assay method for an agent that modulates binding of a IL-TIF/IL-22 to IL-22BP, the method comprising:

contacting an IL-TIF/IL-22 polypeptide or fragment with an IL-22BP polypeptide or fragment thereof and a test substance, under conditions in which in the absence of the test substance being an inhibitor of binding of IL-22BP to IL-TIF/IL-22 the IL-22BP polypeptide or fragment thereof binds the IL-TIF/IL-22 polypeptide or fragment thereof, and

determining binding of the IL22-BP polypeptide or fragment thereof to the IL-TIF/IL-22 polypeptide or fragment thereof.

Modulation of binding identifies the test substance as an agent that modulates binding of IL-22BP to IL-TIF/IL-22.

In a further aspect according to the present invention there is provided an assay method for an agent that modulates biological activity of IL-22BP of the invention, the method comprising

providing an IL-22BP polypeptide under test conditions, and

determining IL-22BP activity in the presence and absence of a test substance.

In different assays of the invention, interaction and/or binding and/or activity of components in the presence of a test compound or substance may be compared with the interaction and/or binding and/or activity in comparable reaction medium and conditions in the absence of a test compound. A test compound able to modulate the interaction and/or activity may be identified. The skilled person is well aware of appropriate experimental controls to perform when conducting assay methods.

Compounds which may be screened may be natural or synthetic chemical compounds used in drug screening programmes. Extracts of plants, microbes or other organisms, which contain several characterised or uncharacterised components may also be used.

Also, combinatorial library technology provides an efficient way of testing a potentially vast number of different substances for ability to modulate an interaction.

Such libraries and their use are known in the art, for all manner of natural products, small molecules and peptides, among others.

The use of peptide libraries may be preferred in certain circumstances. The potential for binding between polypeptide chains of receptors of the invention to be inhibited by means of peptide fragments of the polypeptide chains has been mentioned already. Such peptide fragments may consist of for example 10-40 amino acids, e.g. about 10, about 20, about 30 or about 40 amino acids, or about 10-20, 20-30 or 30-40 amino acids. These may be synthesized recombinantly, chemically or synthetically using available techniques.

In any assay method according to the invention, the amount of test substance or compound which may be added to an assay of the invention will normally be determined by trial and error depending upon the type of compound used. Even a molecule which has a weak effect may be a useful lead compound for further investigation and development.

A screening or assay method may include purifying and/or isolating a test compound and/or substance of interest from a mixture or extract, i.e. reducing the content of at least one component of the mixture or extract, e.g. a component with which the test substance is naturally associated. The screening or assay method may include determining the ability of one or more fractions of a test mixture or extract to bind to a polypeptide or peptide of the invention or determining the ability of one or more fractions of a test mixture or extract to bind to or affect the activity of IL-TIF/IL-22.

The purifying and/or isolation may employ any method known to those skilled in the art, for instance using an antibody molecule.

In addition to their use in purifying polypeptide chains and receptors of the invention, antibody molecules represent themselves a further class of potential modulators of receptor function.

Irrespective of the nature of the test compounds being tested in an assay method of the invention, interaction or binding between substances, e.g. an IL-22BP polypeptide and IL-TIF/IL-22, may be determined by any number of techniques available in the art,

qualitative or quantitative. They include techniques such as radioimmunoassay, co-immunoprecipitation, scintillation proximity assay and ELISA methods.

Binding of one component to another may be studied by labeling either one with a detectable label and bringing it into contact with the other which may have been immobilized on a solid support. Suitable detectable labels, especially for peptidyl substances include  $^{35}\text{S}$ -methionine which may be incorporated into recombinantly produced peptides and polypeptides. Recombinantly produced peptides and polypeptides may also be expressed as fusion proteins containing an epitope which can be labeled with an antibody.

The polypeptide or peptide which is immobilized on a solid support may be immobilized using an antibody against that polypeptide bound to a solid support or via other technologies which are known *per se*. A preferred *in vitro* interaction may utilize a fusion peptide including glutathione-S-transferase (GST). This may be immobilized on glutathione agarose beads. In an *in vitro* assay format of the type described above a test modulator can be assayed by determining its ability to diminish the amount of labeled peptide or polypeptide (e.g. labeled IL-TIF/IL-22) which binds to the immobilized GST-fusion peptide (e.g. immobilized fusion peptide of GST and IL-22BP or fragment thereof). This may be determined by fractionating the glutathione-agarose beads by SDS-polyacrylamide gel electrophoresis. Alternatively, the beads may be rinsed to remove unbound peptide or polypeptide and the amount of peptide or polypeptide which has bound can be determined by counting the amount of label present in, for example, a suitable scintillation counter.

Binding or interaction of two components may also be determined using a two-hybrid assay.

For example, an IL-22BP polypeptide of the invention may be fused to a DNA binding domain such as that of the yeast transcription factor GAL4. The GAL4 transcription factor includes two functional domains. These domains are the DNA binding domain (GAL4DBD) and the GAL4 transcriptional activation domain (GAL4TAD). By fusing a first polypeptide component of the assay to one of those

domains, and a second polypeptide component of the assay to the respective counterpart, a functional GAL4 transcription factor is restored only when the two polypeptides interact. Thus, interaction of these polypeptides may be measured by the use of a reporter gene linked to a GAL4 DNA binding site which is capable of activating transcription of said reporter gene.

This two hybrid assay format is described by Fields and Song, 1989, Nature 340: 245-246. It can be used in both mammalian cells and in yeast. Other combinations of DNA binding domain and transcriptional activation domain are available in the art and may be preferred, such as the LexA DNA binding domain and the VP60 transcriptional activation domain.

The precise format of any of the screening or assay methods of the present invention may be varied by those of skill in the art using routine skill and knowledge. The skilled person is well aware of the need to employ appropriate control experiments.

Performance of an assay method according to the present invention may be followed by isolation and/or manufacture and/or use of a compound, substance or molecule which tests positive for ability to modulate the relevant interaction or affect the relevant biological function or activity. Following identification of a suitable agent, it may be investigated further, and may be modified or derivatized to alter one or more properties, without abolishing its ability to modulate the relevant interaction or affect the relevant biological function. For instance, a single chain Fv antibody molecule may be reformatted into a whole antibody comprising antibody constant regions, e.g. an IgG antibody. Any peptidyl molecule may be modified by addition, substitution, insertion or deletion of one or more amino acids, or by joining of an addition moiety or protein domain. An active agent may be subject to molecular modelling *in silico* and one or more mimetics of the originally identified agent may be created.

Furthermore, an active agent of the invention may be manufactured and/or used in preparation, i.e. manufacture or formulation, of a composition such as a medicament, pharmaceutical composition or drug. These may be administered to individuals, as discussed.

A compound, whether a peptide, antibody, small molecule or other substance found to have the ability to affect binding between polypeptide chains of a receptor of the invention or binding of such a receptor to a ligand has therapeutic and other potential in a number of contexts. For therapeutic treatment such a compound may be used in combination with any other active substance.

Generally, such a substance identified according to the present invention and to be subsequently used is provided in an isolated and/or purified form, i.e. substantially pure. This may include being in a composition where it represents at least about 90% active ingredient, more preferably at least about 95%, more preferably at least about 98%. Such a composition may, however, include inert carrier materials or other pharmaceutically and physiologically acceptable excipients. Thus, a composition may consist of the active ingredient obtained using the invention, and an inert carrier. Furthermore, a composition according to the present invention may include in addition to an modulator compound as disclosed, one or more other molecules of therapeutic use.

Also a part of this invention is a method for determining the presence of the receptor-like antagonist of this invention in a tissue or cell sample comprising contacting said sample with an antibody specific for said receptor-like antagonist and determining binding therebetween. Methods for determining the binding of an antibody and its antigen are well known to those of skill in the art and need not be elaborated herein.

The receptor-like binding protein of this invention may also be used to determine the presence of IL-TIF/IL-22 in a sample by, e.g., labeling said receptor-like binding protein and then contacting said sample with said receptor-like antagonist and determining binding therebetween wherein said binding is indicative of the presence of IL-TIF. Alternatively, one may determine the presence of IL-TIF/IL-22 in a sample by treating a cell line that is responsive to IL-TIF/IL-22 to two aliquots of said sample, one containing the receptor-like binding protein and one without the receptor-like binding protein, then measuring and comparing the response of said responsive cell to the two aliquots wherein a difference in response to the two aliquots is indicative of the presence of IL-TIF/IL-22. In the alternative, cells that are responsive to IL-TIF/IL-22 can be used

in such assays. To elaborate, cells which show some type of response to IL-TIF/IL-22, such as increased STAT activation or acute phase reactant production, can be used to screen for presence and/or amount of IL-22BP in a sample. For example, assuming that the cell is incubated in the sample in question together with IL-TIF/IL-22, any observed change in the response, such as a decrease in STAT activation or acute phase reactant production, is indicative of IL-22 BP in said sample.

The soluble IL-TIF/IL-22 binding proteins described herein are further examples of soluble, cytokine receptors generated in vivo. See, e.g. Rose-John, et al., *Biochem J.* 300: 281 (1994); Fernandez-Botran, et al., *Adv. Immunol* 63:269 (1996). Heaney, et al., *Blood* 87: 845 (1996). Soluble cytokine receptors compete with cell surface receptors for binding to free or unbound cytokine molecules. With the exception of IL-6R, this binding prevents cytokines from reaching the cell membrane and generating a signal. The binding is generally reversible, leading to temporary sequestration of the cytokine from membrane receptors. Soluble cytokine receptors also enhance the activity of cytokines by modifying their stability, decreasing proteolytic degradation, or reducing clearance. Such functions, i.e., as cytokine carriers *in vivo*, are seen to help potentiate the systemic effect of cytokines, with the antagonistic effect being pertinent to paracrine activities.

Other features of the invention will be clear to the artisan and need not be discussed further.

The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, it being recognized that various modifications are possible within the scope of the invention.

**WE CLAIM:**

1. An isolated nucleic acid molecule which encodes a soluble protein which binds to IL-TIF/IL-22, wherein the complementary nucleotide sequence of said isolated nucleic acid molecule, hybridizes, under stringent conditions, to SEQ ID NO: 5 or SEQ ID NO: 10.
2. The isolated nucleic acid molecule of claim 1, wherein said isolated nucleic acid molecule encodes a protein, the amino acid sequence of which is set forth in SEQ ID NO: 6 or SEQ ID NO: 11.
3. The isolated nucleic acid molecule of claim 1, comprising the nucleotide sequence set forth at SEQ ID NO: 5 or SEQ ID NO: 10.
4. Expression vector comprising the isolated nucleic acid molecule of claim 1, operably linked to a promoter.
5. Expression vector comprising the isolated nucleic acid molecule of claim 2, operably linked to a promoter.
6. Expression vector comprising the isolated nucleic acid molecule of claim 3, operably linked to a promoter.
7. Recombinant cell line or cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 1.
8. Recombinant cell line or cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 2.
9. Recombinant cell line or cell strain, transformed or transfected with the isolated nucleic acid molecule of claim 3.
10. Recombinant cell line or cell strain, transformed or transfected with the expression vector of claim 4.
11. Recombinant cell line or cell strain, transformed or transfected with the expression vector of claim 5.
12. Recombinant cell line or cell strain, transformed or transfected with the expression vector of claim 6.



13. Isolated, soluble binding protein which binds to IL-TIF/IL-22, having a molecular weight of from about 23 to about 40 kilodaltons, as determined by SDS-PAGE.
14. The isolated soluble protein of claim 13, comprising the amino acid sequence set forth at SEQ ID NO: 6 or SEQ ID NO: 11.
15. A method for inhibiting effect of IL-TIF/IL-22 on a cell, comprising contacting said IL-TIF/IL-22 with the soluble protein of claim 13 in an amount sufficient to bind to and antagonize said IL-TIF/IL-22.
16. A method for determining if IL-TIF/IL-22 is present in a sample, comprising contacting said sample with the protein of claim 13, and determining binding of said protein to IL-TIF/IL-22 as a determination of IL-TIF/IL-22 in said sample.
17. A method for producing a soluble, IL-22/IL-TIF binding protein comprising transforming or transfecting a cell with the isolated nucleic acid molecule of claim 1, culturing the thus transformed or transfected cell to produce said soluble binding protein, and isolating it from said cell.
18. A method for producing a soluble, IL-TIF/IL-22 binding protein, comprising transforming or transfecting a cell with the expression vector of claim 4, culturing the thus transformed or transfected cell to produce said soluble protein, and isolating it from said cell.
19. The isolated, soluble binding protein of claim 13, further comprising a detectable label.
20. The isolated, soluble binding protein of claim 13, wherein said soluble binding protein is an antagonist for IL-TIF/IL-22.
21. Isolated antibody which specifically binds to the binding protein of claim 13.
22. The antibody of claim 21, wherein said antibody is monoclonal antibody.
23. Hybridoma cell line which produces the monoclonal antibody of claim 22.
24. A method for determining presence of a soluble protein which binds to IL-TIF/IL-22, comprising contacting said sample with the antibody of claim 21, and determining binding of said antibody to said soluble, binding protein as a determination of presence of said soluble, binding protein in said sample.

25. The method of claim 24, wherein said antibody is labeled with a detectable label.
26. A method for determining expression of nucleic acid molecule which encodes a protein antagonist of IL-TIF/IL-22 binding protein in a sample, comprising contacting said sample with an oligonucleotide which hybridizes specifically, under stringent conditions to the nucleotide sequence of SEQ ID NO: 5 or SEQ ID NO: 10, hybridization thereto being indicative of expression of said nucleic acid molecule.
27. An isolated oligonucleotide consisting of from 17 to 100 contiguous nucleotides of SEQ ID NO: 5 or SEQ ID NO: 10.
28. An isolated protein which binds to IL-TIF/IL-22, produced by the method of claim 17.
29. An isolated protein which binds to IL-TIF/IL-22, produced by the method of claim 18.
30. A method for inhibiting binding of IL-TIF/IL-22 to a binding partner, comprising adding an amount of the isolated binding protein of claim 13 to a sample containing IL-TIF/IL-ss and a binding partner therefor, sufficient to inhibit said binding.
31. A method according to claim 15 that takes place *in vitro*.
32. A method according to claim 17 or claim 18 further comprising testing or confirming ability of the soluble binding protein to bind IL-TIF/IL-22.
33. A method of obtaining an antibody molecules specific for the soluble binding protein of claim 13 or claim 14, the method comprising bringing a population or panel of antibody molecules of diverse binding specificity into contact with the soluble binding protein or an antigenic fragment thereof, selecting one or more antibody molecules that bind the protein, and testing said antibody molecules for binding specificity for the soluble binding protein, whereby an antibody molecule specific for the soluble binding protein is obtained.
34. A method according to claim 33 further comprising testing the antibody molecule for ability to inhibit binding of IL-22BP to IL-TIF/IL-22.

35. Use of a soluble binding protein according to claim 13 or claim 14 in the manufacture of a medicament for treating an IL-22-mediated disease.
36. An assay method for an agent that modulates binding of a soluble binding protein according to claim 13 or claim 14 to IL-TIF/IL-22, the method comprising:  
contacting an IL-TIF/IL-22 polypeptide or fragment with the protein of claim 13 or claim 14 or fragment thereof and a test substance, under conditions in which in the absence of the test substance being an inhibitor of binding of the protein to IL-TIF/IL-22 the protein or fragment thereof binds the IL-TIF/IL-22 polypeptide or fragment thereof,  
  
determining binding of the IL22-BP polypeptide or fragment thereof to the IL-TIF/IL-22 polypeptide or fragment thereof, and  
  
identifying an agent that modulates said binding.
37. A method according to claim 36 further comprising testing the agent that modulates said binding for ability to modulate an IL-22-mediated activity.
38. A method according to claim 36 or claim 37 wherein the agent that modulates said binding is formulated into a composition comprising at least one additional component.
39. A method according to claim 39 further comprising use of the agent that modulates said binding in the manufacture of a medicament for treating an IL-22-mediated disorder.
40. A method according to claim 36 or claim 37 further comprising contacting IL-TIF/IL-22 and/or IL-22 binding protein with said agent that modulates said binding.
41. A method according to claim 40 that takes place *in vitro*.
42. A method according to claim 40 that comprises administering to an individual said agent that modulates binding.
43. A method according to any one of claims 36 to 42 wherein said agent that modulates said binding is an antibody molecule.

44. A method according to any one of claims 36 to 42 wherein said agent that modulates said binding is a peptide fragment of IL-TIF/IL-22 or the soluble binding protein of claim 13 or claim 14.

<110> Renault, Jean-Christophe  
Dumoutier, Laure

<120> Isolated Nucleic Acid Molecules Which Encode A Soluble IL-TIF/IL-22 Receptor or  
Binding Protein Which Binds to IL-TIF/IL-22, And Uses Thereof

<130> LUD 5684.3PCT

<140>

<141>

<150>US09/919,162

<151>2001-31-07

<150> US 60/245,495

<151> 2000-03-11

<150> US60/234,583

<151> 2000-22-09

<160> 11

<210> 1

<211> 21

<212> DNA

<213> Homo sapiens

<400> 1

agggtacaat ttcagtcccg a 21

<210> 2

<211> 21

<212> DNA

<213> Homo sapiens

<400> 2

cggcgcatg ctccattctg a 21

<210> 3

<211> 28

<212> DNA

<213> Homo sapiens

<400> 3

tgaacagtca cactcgagac catgatgc 28

<210> 4  
 <211> 25  
 <212> DNA  
 <213> Homo sapiens

<400> 4

catcctgttc tcgaggagct ttaga 25

<210> 5  
 <211> 2271  
 <212> DNA  
 <213> Homo sapiens

<400> 5

ctgcctaaa cccgggagtg attgtctgtt tgtggatttt acagtttct ctttggctct	60
gagctggta aaaggaacac tggttgcctg aacagtcaca ctgcaacca tgaatcctaa	120
acattgcttt ctaggcttcc tcatcagttt ctctcttact ggtgtagcag gaactcagtc	180
aacgcatgag tctctgaagc ctgagagggt acaatttcag tcccgaaatt ttcacaacat	240
tttgaatgg cagcctggga gggcacttac tggcaacagc agtgtctatt ttgtgcagta	300
caaaatatat ggacagagac aatggaaaaa taaagaagac tgttggggta ctcaagaact	360
ctctgtgac cttaccagtg aaacctcaga catacaggaa ccttattacg ggagggtgag	420
ggcggcctcg gctgggagct acicagaatg gagcatgacg ccgcgggtca ctccctggtg	480
ggaaacaaaa atagatcctc cagtcagtaa tataacccaa gtcaatggct ctttgttgg	540
aatttccat gctccaaatt taccatatag atacaaaag gaaaaaatg tatctataga	600
agattactat gaactactat accgagtttt tataattaac aattcactag aaaaggagca	660
aaaggtttat gaaggggctc acagagcggg tgaattgaa gctctaacac cacactccag	720
ctactgtgta gtggctgaaa tatatcagcc catgtagac agaagaagtc agagaagtga	780
agagagatgt gtggaaattc catgacttgt ggaatttggc attcagcaat gtggaaattc	840
taaagctccc tgagaacagg atgactcgtg ttgaaggat cttatttaa attgttttg	900
tattttctta aagcaatatt cactgttaca ccttggggac ttctttgtt atccattctt	960
ttatccttta tatttcattt gtaactata ttgaacgac attcccccg aaaaattgaa	1020
atgtaaagat gaggcagaga ataaagtgt ctatgaaat cagaacttta ttctgaatg	1080
taacatccct aataacaacc ttattcttc taatacagca aaataaaaat ttaacaacca	1140
aggaatagta ttaagaaaa tgttgaaata attttttaa aatagcatta cagactgagg	1200
cggctctgaa gcaatgggtt ttactctct tattgagcca attaaattga cattgctttg	1260
acaattttaa acttctataa aggtgaatat ttctcataca ttctatttt atatgaatat	1320
actttttata tatttattat tattaatat ttctacttaa tgaatcaaaa tttgtttta	1380
aagtctactt tatgtaaata agaacagggt ttggggaaaa aaatcttatg atttctggat	1440
tgatatctga attaaaaa tcaacaacaa ggaagtctgc tctgtacaat tgcctctcat	1500
ttaaagata tattaagctt ttctttctg ttgtttttg tttgtttag ttttaatcc	1560
tgtcttagaa gaacttatct ttattctcaa aattaaatgt aatttttta gtgacaaaga	1620
agaaaggaaa cctcattact caatccttct ggccaagagt gtcttgcttg tggcgccttc	1680
ctcatctcta tataggagga tccatgaat gatggtttat tgggaactgc tggggtcgac	1740
cccatacaga gaactcagct tgaagctgga agcacacagt gggtagcagg agaaggaccg	1800
gtgttggtag gtgcctacag agactataga gctagacaaa gccctccaaa ctggccctc	1860

ctgtcactg cctctcctga gtagaaatct ggtgacctaa ggctcagtgt ggtcaacaga	1920
aagctgcctt cttcacttga ggctaagtct tcatatatgt ttaaggttgt cttttagtg	1980
aggagataca taccagagaa catttgtaca attcccatg aaaattgctc caaagttgat	2040
aacaatatag tcgggtcttc tagttatatg caagtactca gtgataaatg gattaaaaaa	2100
tattcagaaa tgtattgggg ggtggaggag aataagaggc agagcaagag ctagagaatt	2160
ggtttcctg cttccctgta tgctcagaaa acattgatt gagcatagac gcagagactg	2220
aaaaaaaaat ttactttgat ctctgtttt gaattcttat tatttatatt t	2271

&lt;210&gt; 6

&lt;211&gt; 231

&lt;212&gt; PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 6

Met Met Pro Lys His Cys Phe Leu Gly Phe Leu Ile Ser Phe Phe Leu	
	5 10 15
Thr Gly Val Ala Gly Thr Gln Ser Thr His Glu Ser Leu Lys Pro Gln	
	20 25 30
Arg Val Gln Phe Gln Ser Arg Asn Phe His Asn Ile Leu Gln Trp Gln	
	35 40 45
Pro Gly Arg Ala Leu Thr Gly Asn Ser Ser Val Tyr Phe Val Gln Tyr	
	50 55 60
Lys Ile Tyr Gly Gln Arg Gln Trp Lys Asn Lys Glu Asp Cys Trp Gly	
	65 70 75 80
Thr Gln Glu Leu Ser Cys Asp Leu Thr Ser Glu Thr Ser Asp Ile Gln	
	85 90 95
Glu Pro Tyr Tyr Gly Arg Val Arg Ala Ala Ser Ala Gly Ser Tyr Ser	
	100 105 110
Glu Trp Ser Met Thr Pro Arg Phe Thr Pro Trp Trp Glu Thr Lys Ile	
	115 120 125
Asp Pro Pro Val Met Asn Ile Thr Gln Val Asn Gly Ser Leu Leu Val	
	130 135 140
Ile Leu His Ala Pro Asn Leu Pro Tyr Arg Tyr Gln Lys Glu Lys Asn	
	145 150 155 160
Val Ser Ile Glu Asp Tyr Tyr Glu Leu Leu Tyr Arg Val Phe Ile Ile	
	165 170 175
Asn Asn Ser Leu Glu Lys Glu Gln Lys Val Tyr Glu Gly Ala His Arg	
	180 185 190
Ala Val Glu Ile Glu Ala Leu Thr Pro His Ser Ser Tyr Cys Val Val	
	195 200 205
Ala Glu Ile Tyr Gln Pro Met Leu Asp Arg Arg Ser Gln Arg Ser Glu	
	210 215 220
Glu Arg Cys Val Glu Ile Pro	
	225 230

<210> 7  
 <211> 37  
 <212> DNA  
 <213> Homo sapiens

<400> 7  
 ccaacttcca tgatcaatgg aatttcaca catctct 37

<210> 8  
 <211> 33  
 <212> DNA  
 <213> Homo sapiens

<400> 8  
 aagactgagt tgatcaagag aatcgagcct aga 33

<210> 9  
 <211> 27  
 <212> DNA  
 <213> Homo sapiens

<400> 9  
 aatgtctaga tgctgtctc atttacc 27

<210> 10  
 <211> 2366  
 <212> DNA  
 <213> Homo sapiens

<400> 10	
ctgccttaaa cccgggagtg attgtctgtt tgtggatttt acagtttctt ctttggtcct	60
gagctgggta aaaggaacac tgggtgcctg aacagtcaca ctgcaacca tgatgcctaa	120
acattgcttt ctaggcttcc tcatcagttt cttccttact ggtgtagcag gaactcagtc	180
aacgcatgag tctctgaagc ctcagagggt acaatttcag tcccgaattt ttcacaacat	240
tttgcaatgg cagcctggga gggcacttac tggcaacagc agtgtctatt ttgtgcagta	300
caaaatcatg ttctcatgca gcatgaaaag ctctcaccag agccaagtgg atgcttggca	360
gcacatttct tgtaacttcc caggctgcag aacattggct aaatatggac agagacaatg	420
gaaaaataaa gaagactgtt ggggtactca agaactctct tgtgacctta ccagtgaac	480
ctcagacata caggaacctt attacgggag ggtgagggcg gcctcggctg ggagctactca	540
gaatggagca tgacgccgcg gttcactccc tgggtggaaa caaaaataga tcctccagtc	600
atgaatataa cccaagtcaa tggctctttg ttgtaattc tccatgctcc aaatttacca	660
tatagatacc aaaaggaaaa aaatgtatct atagaagatt actatgaact actataccga	720
gtttttataa ttaacaattc actagaaaag gagcaaaagg tttatgaagg ggctcacaga	780
gcgggttgaat tgaagctct aacaccacac tccagctact gtgtagtggc tgaatatat	840



cagcccatgt tagacagaag aagtcagaga agtgaagaga gatgtgtgga aattccatga	900
cttgtggaat ttggcattca gcaatgtgga aattctaaag ctccctgaga acaggatgac	960
tcgtgttga aggatcttat ttaaaattgt tttgtattt tcttaaagca atattcactg	1020
ttacaccttg gggacttctt tgtttatcca tcttttatc ctttatatt catttgtaa	1080
ctatatttga acgacattcc ccccgaaaaa ttgaaatga aagatgaggc agagaataaa	1140
gtgttctatg aaattcagaa ctttatttct gaatgtaaca tcctaataa caaccttcat	1200
tcttctaata cagcaaaata aaaatttaac aaccaaggaa tagtatttaa gaaaatgttg	1260
aaataathtt tttaaaatag cattacagac tgaggcggtc ctgaagcaat ggttttcac	1320
tctctattg agccaattaa atgacattg ctttgacaat ttaaaacttc tataaagggtg	1380
aataattttc atacatttct attttatag aatatacttt ttatatatt attattatta	1440
aataatttcta ctaatgaat caaaatttg ttttaaagtc tactttatgt aaataagaac	1500
aggttttggg gaaaaaaatc ttatgatttc tggattgata tctgaattaa aactatcaac	1560
aacaagggaag tctgctctgt acaattgtcc ctcatthaa agatatatta agcttttctt	1620
ttctgtttgt tttgttttg tttagtttt aatcctgtct tagaagaact tatctttatt	1680
ctcaaaatta aatgtaattt ttttagtgac aaagaagaaa ggaaacctca ttactcaatc	1740
cttctggcca agagtgtctt gcttgtggcg ccttcctcat ctctatatag gaggatccca	1800
tgaatgatgg ttattggga actgctgggg tcgaccccat acagagaact cagcttgaag	1860
ctggaagcac acagtgggta gcaggagaag gaccgggtgt ggtagggtcc tacagagact	1920
atagagctag acaaagccct ccaaactggc ccctcctgct cactgcctct cctgagtaga	1980
aatctggtga cctaaggctc agtgtgtgtca acagaaagct gccttctca ctgaggcta	2040
agtcctcata tatgtttaag gttgtcttc tagtgaggag atacatatca gagaacattt	2100
gtacaattcc ccatgaaaat tgctccaaag ttgataacaa tatagtcggt gcttctagtt	2160
atatgcaagt actcagtgat aaatggatta aaaaatattc agaatgtat tgggggggtgg	2220
aggagaataa gaggcagagc aagagctaga gaattggttt ccttgcttcc ctgtatgctc	2280
agaaaacatt gatttgagca tagacgcaga gactgaaaaa aaaatttact ttgatctctg	2340
ttttgaatt cttattattt atattt	2366

&lt;210&gt; 11

&lt;212&gt; 263

&lt;212&gt;PRT

&lt;213&gt; Homo sapiens

&lt;400&gt; 11

Met Met Pro Lys His Cys Phe Leu Gly Phe Leu Ile Ser Phe Phe Leu	
	5 10 15
Thr Gly Val Ala Gly Thr Gln Ser Thr His Glu Ser Leu Lys Pro Gln	
	20 25 30
Arg Val Gln Phe Gln Ser Arg Asn Phe His Asn Ile Leu Gln Trp Gln	
	35 40 45
Pro Gly Arg Ala Leu Thr Gly Asn Ser Ser Val Tyr Phe Val Gln Tyr	
	50 55 60

Lys Ile Met Phe Ser Cys Ser Met Lys Ser Ser His Gln Ser Gln Val  
 65                      70                      75                      80  
 Asp Ala Trp Gln His Ile Ser Cys Asn Phe Pro Gly Cys Arg Thr Leu  
                     85                      90                      95  
 Ala Lys Tyr Gly Gln Arg Gln Trp Lys Asn Lys Glu Asp Cys Trp Gly  
                     100                      105                      110  
 Thr Gln Glu Leu Ser Cys Asp Leu Thr Ser Glu Thr Ser Asp Ile Gln  
                     115                      120                      125  
 Glu Pro Tyr Tyr Gly Arg Val Arg Ala Ala Ser Ala Gly Ser Tyr Ser  
                     130                      135                      140  
 Glu Trp Ser Met Thr Pro Arg Phe Thr Pro Trp Trp Glu Thr Lys Ile  
 145                      150                      155                      160  
 Asp Pro Pro Val Met Asn Ile Thr Gln Val Asn Gly Ser Leu Leu Val  
                     165                      170                      175  
 Ile Leu His Ala Pro Asn Leu Pro Tyr Arg Tyr Gln Lys Glu Lys Asn  
                     180                      185                      190  
 Val Ser Ile Glu Asp Tyr Tyr Glu Leu Leu Tyr Arg Val Phe Ile Ile Asn  
                     195                      200                      205  
 Asn Ser Leu Glu Lys Glu Gln Lys Val Tyr Glu Gly Ala His Arg Ala Val  
 210                      215                      220                      225  
 Glu Ile Glu Ala Leu Thr Pro His Ser Ser Tyr Cys Val Val Ala Glu  
                     230                      235                      240  
 Ile Tyr Gln Pro Met Leu Asp Arg Arg Ser Gln Arg Ser Glu Glu Arg  
                     245                      250                      255  
 Cys Val Glu Ile Pro  
 260